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Adoptive immunotherapy with the human MHC non-restricted cytotoxic T cell line TALL-104 has induced long-term complete or partial remissions in tumor bearing mice and pets with spontaneous tumors. The purpose of this study was to evaluate the safety of lethally irradiated TALL-104 cells in patients with metastatic breast cancer who had relapsed after conventional therapies. This was a single center, dose escalating study with 5 dose levels tested from 10^6 to 10^8 cells/kg of body weight. The 15 patients enrolled (3 patients/dose) received 5 intravenous daily injections of irradiated TALL-104 cells followed by 2-day monthly boosts at the same dose until disease progression. No clinical toxicity was observed even at the highest dose. Aside from individual variations, tests monitoring immunological parameters before and after cell administrations showed a common trend, including: a) a transient absolute and relative monocytosis and eosinophilia; b) slight neutropenia; and c) absence of humoral and cellular responses to TALL-104 cells. Some patients had stable disease for several months and one patient had a marginal clinical response to TALL-104 cells as shown by a decrease in size of liver metastases and ascites. The overall data indicate that TALL-104 cells are well tolerated and might prove effective in adjuvant therapy of breast cancer.

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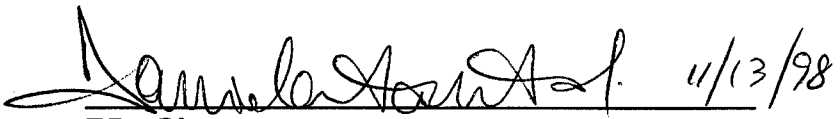

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Introduction

The MHC non-restricted human TALL-104 killer cell line displays potent anti-tumor effects in mice with implanted tumors and in pets (dogs and cats) with spontaneously occurring malignancies (1-4). Mammary tumors are particularly sensitive to TALL-104 cell lysis both in vitro and in animals (1,5). An ongoing phase II clinical trial is testing the ability of TALL-104 cells to induce long-term disease-free survival in dogs and cats with malignant mammary tumors. Five canine and 19 feline patients at high risk of relapse after surgical removal of the primary tumors and metastatic lymph nodes received conventional adjuvant chemotherapy and irradiated TALL-104 cells (10^8/kg) systemically for 5 consecutive days, followed by 4 monthly boosts. No clinical toxicities developed during cell therapy in any of the animals and a strong correlation was found between the patients clinical and immunological responses. One dog died 16 months later from causes unrelated to cancer while the other 4 dogs are still disease-free up to 24 months later, despite their poor prognosis (relapse rate = 100% in 12 months). In the feline population, TALL-104 cell therapy resulted in significant increases in disease-free intervals: only 3 out of 19 cats relapsed and the remaining 16 cats have been disease-free for 2 years.

We have recently investigated TALL-104 cell trafficking in healthy and tumor bearing mice to determine the influence of different factors on in vivo cell distribution patterns (6). In healthy mice ^{111}In-labeled TALL-104 cells, injected i.v., localized primarily in the lungs 2 hr after injection and then redistributed to liver, spleen, and kidneys by 24 hr. In mice bearing human breast tumor biopsies, TALL-104 cells were shown to consistently traffic to tumor site, showing a preferential accumulation in organs with metastases, such as lymph nodes and lungs (1,6).

The purpose of the present study, supported by the U.S. Army, was to evaluate the safety of and establish the maximal tolerated dose (MTD) of lethally irradiated TALL-104 cells in patients with metastatic breast cancer whose disease had not responded to conventional cancer therapy. Supportive laboratory objectives included a) to determine in vitro susceptibility of patients' tumor biopsies (when available) to TALL-104 cell killing; b) to measure plasmatic levels of cytokines in patients receiving TALL-104 cell injections (as a predictor of response and/or toxicity); c) to measure patients peripheral blood lymphocytes for cytotoxic activity in vitro against NK-sensitive and -resistant targets and autologous and allogeneic breast tumors; d) to monitor the development of immune responses (both humoral and cellular) against TALL-104 cells.

Body

This investigation was designed as a single center, dose-escalating study, with 5 dose levels to be tested: 10^6, 3 x 10^6, 10^7, 3 x 10^7, and 10^8 cells/kg of body weight. Each dose level was to be tested in three patients. No dose escalation was proposed within the same patient. Each patient received a first cycle consisting of 5 consecutive days of intravenous (i.v.) injections of TALL-104 cells (at the dose level correspondent to the entry number) followed by 2-day monthly boosts (at the same dose level) until disease progression. The total number of subjects planned to be enrolled in the study was 15 to 20. To date (November 13, 1998), 15 patients have been enrolled (Table 1). One patient (#011) dropped out of the investigation before completion of the study despite her objective clinical response to the cells. The highest dose level of TALL-104 cells to be tested (10^8/kg) was reached without any significant clinical toxicity and/or result (Table 1).

Clinical and Laboratory Findings

1. No serious (life threatening) adverse experiences were observed during and/or after TALL-104 cell injections at any dose level tested.

2. One patient (UPCC-5196 #006) died of rapidly progressive disease within 30 days from the beginning of her first 5 day-cycle (i.e., before the monthly boost).

3. No patient dropped out during the course of the investigation in association with any adverse experience (related or not to the cell administration). However, patient #011 withdrew from the study for personal reasons after receiving the first cycle of 5-day infusions. A minor clinical response was revealed by MRI in this patient by her oncologist.

4. Results of laboratory tests performed on patients blood samples to monitor their immune parameters showed a common trend, specifically: a) a significant increase in the absolute number and percentage of peripheral blood monocytes and eosinophils (Table 1); b) an increase in cytotoxic activity in vitro against the NK sensitive tumor target K562 during cell cycles; c) peaks of serum cytokines during cell treatment in patients who also showed some temporary biological response to TALL-104 cells; d) absence of significant humoral and/or cytotoxic T-cell immunity against TALL-104 cells (up to 2-month follow-up); and e) absence of TALL-104 cell persistence in the blood (up to 2-month follow-up). The latter test has been performed only in the first 3 patients who received the lowest dose of 10^6/kg. Further testing in other patients is ongoing.

5. Standard in vitro ^{51}Cr-release assays (18 hr) were performed to test the susceptibility of tumor cells from patient biopsy specimens (when available) to TALL-104 cell lysis. Results, shown in Table 2, indicate that all four samples tested were killed by TALL-104 cells at various extents. Tumor cells from some patients produced cytokines (mostly TNF-α or IL-10), as measured by ELISA (Table 2). Cytokines such as TNF-β, GM-CSF, and IFN-γ were also found in the supernatants of tumor/TALL-104 mixed cultures from patients #008 (Table 2).

6. Tumor biopsies from patients #003, 008, 009, and 015 were implanted as whole 5x5 mm fragments in SCID mice, according to established techniques (1,7). As of November 1998, one sample (from patient #009) has grown successfully in 50% of the implanted mice (Table 3). Because in a previous study (7) we have shown that tumor uptake might occur after follow-up periods close to one year, the in vivo growth of some of the implanted tumor fragments might be detectable in the months to come. The engrafted tumor masses will be removed and implanted again in other groups of SCID mice to develop metastatic models for TALL-104 therapy as single agent and in combination with chemotherapeutic drugs, as described in the proposal.

Conclusions

Results from this phase I trial indicated that TALL-104 cells are well tolerated by terminally ill breast cancer patients up to the maximal intended dose. In addition, encouraging clinical responses were observed, including stabilization of disease in some patients, which warrant further studies to evaluate the optimal regimen of TALL-104 cell administration resulting in significant anti-tumor effects.

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Appendices

Table 1. Phase I trial of TALL-104 cells in metastatic breast cancer patients (UPCC-5196)

Table 2. TALL-104 cell lysis of breast tumor cells from patient's biopsies

Table 3. Engraftment of breast tumor biopsies in SCID mice

Table 1. Phase I trial of TALL-104 cells in metastatic breast cancer patients (UPCC-5196)

UPCC-5196 Patient code (sex, age)	Diagnosis	TALL-104 cell dose/kg (cycle)[a]	Clinical toxicity (cycle)	Laboratory changes[b]	Clinical response[c] (cycles)
001 (F,35)	Infiltrating ductal carcinoma	(I) 10^6 (II) 10^6 (III) 10^6	Bilateral erythema in ulnar eminance (I)	↑ Monocytes ↑ Lymphocytes ↓ Neutrophils ↑ Gamma GT ↓ LDH	SD (I, II) (transient decrease in bone pain) PD (II)
002 (F,60)	Infiltrating ductal carcinoma	(I) 10^6	None	↓ WBC and neutrophils ↑ Monocytes	PD
003 (F,63)	Infiltrating lobular carcinoma	(I) 10^6 (II) 10^6 (III) 10^6 (IV) 10^6	None	Occasional hypocalcemia and elevated SGOT	SD (I, II, III) PD (IV)
004 (F,37)	Infiltrating ductal carcinoma	(I) 3×10^6	None	↑ Monocytes ↓ WBC	PD
005 (F,52)	Infiltrating ductal carcinoma	(I) 3×10^6 (II) 3×10^6 (III) 3×10^6 (IV) 3×10^6 (V) 3×10^6 (VI) 3×10^6	Dizziness, nausea, taste change[d] (I)	↑ Monocytes ↑ Eosinophils ↑ Lymphocytes ↓ Granulocytes	SD (I→V) PD (VI)
006 (F,48)	Intraductal and infiltrating carcinoma with apocrine features	(I) 3×10^6 (II) 3×10^6	None	↑ Monocytes, ↑ lymphocytes ↑ Eosinophils ↓ WBC ↓ Granulocytes	SD (I) PD (II)
007 (F,43)	Medullary carcinoma	(I) 10^7	Nausea, vomiting, taste change[d]	None	PD
008 (F,47)	Infiltrating ductal carcinoma with medullary features	(I) 10^7	None	None	PD
009 (F,56)	Infiltrating ductal carcinoma	(I) 10^7	None	None	PD
010 (F, 38)	Infiltrating ductal carcinoma	(I) 3×10^7	None	Pending	PD
011 (F,34)	Infiltrating ductal carcinoma	(I) 3×10^7	None	Pending	Marginal response (I) Off study (patient withdrawal)

Table 1. (continued)

UPCC-5196 Patient code (sex, age)	Diagnosis	TALL-104 cell dose/kg (cycle)[a]	Clinical toxicity (cycle)	Laboratory changes[b]	Clinical response[c] (cycles)
012 (F,56)	Infiltrating ductal carcinoma	(I) 3×10^7	None	Pending	PD
013 (F,54)	Infiltrating ductal carcinoma	(I) 10^8 (II) 10^8 (III) 10^8	None	Pending	SD (I→III) ongoing
014 (F,49)	Infiltrating ductal carcinoma	(I) 10^8	None	Pending	PD
015 (F,59)	Infiltrating ductal carcinoma	(I) 10^8	None	Pending	PD

[a]Cycle I = 5 consecutive days of TALL-104 injections; Cycles II–VI = 2-day monthly boosts.
[b]These findings were transient. Values normalized within a week from the last cell injection.
[c]SD = stable disease; PD = progressive disease.
[d]Attributable to the presence of DMSO in the infusion bag.

Table 2. TALL-104 cell lysis of breast tumor cells from patients' biopsies

UPCC-5196 Patient no.	Type of biopsy	Sensitivity to TALL-104 cell lysis (18 hr assay)	Cytokine production	
			by the tumor	upon contact with TALL-104
001	n.a.	n.a.	n.a.	n.a.
002	Skin lesion	Pathology results negative	n.d.*	n.d.*
003	Lymph node	–	n.d.	n.d.
004	n.a.	n.a.	n.a.	n.a
005	n.a.	n.a.	n.a.	n.a.
006	Pleural effusion	n.d.*	TNF-α IL-10	n.a.
007	n.a.	n.a.	n.a.	n.a.
008	Chest wall	++	TNF-α**	IL-10 TNF-α TNF-β GM-CSF IFN-γ
009	Lymph node	+	None	None
010	n.a.	n.a	n.a.	n.a.
011	n.a.	n.a.	n.a.	n.a.
012	Lymph node	+++	Pending	Pending
013	n.a.	n.a.	n.a.	n.a.
014	n.a.	n.a	n.a	n.a.
015	Lymph node	+	Pending	Pending

n.d. – not done; *, not enough material. n.a. – not available

** Note: the chest wall biopsy of patient #008 produced TNF-α by itself, but upon interaction with TALL-104 TNF-α production was significantly diminished.

Table 3. Engraftment of breast tumor biopsies in SCID mice

UPCC-5196 Paient no.	Type of biopsy	No. of SCID mice engrafted	No. of SICD mice with successful engraftment	Follow-up period
003	Lymph node	4	0	9+ months
008	Chest wall	4	0	7+ months
009	Lymph node	4	2	6+ months
015	Lymph node	3	0	7+ months